Table S1: Yeast strains used in these analyses.

BY4741: MATa; his3Δ1; leu2Δ0; lys2Δ0; ura3Δ0

trf4Δ: MATa; his3Δ1; leu2Δ0; lys2Δ0; ura3Δ0, trf4Δ::kanMX

trf5: MATa; his3Δ1; leu2Δ0; lys2Δ0; ura3Δ0, trf5Δ::kanMX4

trf4Δ, GAL::trf5: MATa; his3Δ1; leu2Δ0; lys2Δ0; ura3Δ0, trf4Δ::kanMX4, HisMX6-pGAL-3HA::trf5

rrp6Δ: MATa; his3Δ1; leu2Δ0; lys2Δ0; ura3Δ0, rrp6Δ::natMX

rrp6Δ, trf4Δ: MATa; his3Δ1; leu2Δ0; lys2Δ0; ura3Δ0, trf4Δ::kanMX, rrp6Δ::natMX

rrp6Δ, trf5: MATa; his3Δ1; leu2Δ0; lys2Δ0; ura3Δ0, trf5Δ::kanMX4, rrp6Δ::natMX

GAL::mtr4: MATa; his3Δ1; leu2Δ0; lys2Δ0; ura3Δ0, HisMX6-pGAL-3HA::mtr4

GAL::rrp41: MATa; his3Δ1; leu2Δ0; lys2Δ0; ura3Δ0, HisMX6-pGAL-3HA::rrp41

GAL::rrp44: MATa; his3Δ1; leu2Δ0; lys2Δ0; ura3Δ0, HisMX6-pGAL-3HA::rrp44

TRF5-TAP: MATa; his3Δ1; leu2Δ0; lys2Δ0; ura3Δ0, TRF5-TAP-URA3

TRF5-TAP DADA: MATa; his3Δ1; leu2Δ0; lys2Δ0; ura3Δ0, TRF5 (D233A,D235A)*-TAP-URA3*

TRF5-TAP trf4D: MATa; his3Δ1; leu2Δ0; lys2Δ0; ura3Δ0, trf4Δ::kanMX, TRF5-TAP-URA3

Table S2. Hybridization probes used in this work. Major RNA species detected in Figs. 1-3 are indicated in brackets.

003 ($27SA_2$, 23S pre-rRNA) TGTTACCTCTGGGCCC

004 (20S, 23S pre-rRNA) CGGTTTTAATTGTCCTA

007 (25S) CTCCGCTTATTGATATGC

008 (18S) CATGGCTTAATCTTTGAGAC

015 (5.8S) TTTCGCTGCGTTCTTCATC

041 (5S) CTACTCGGTCAGGCTC

020 (7S, 5.8S+30, 6S, 27S pre-rRNA) TGAGAAGGAAATGACGCT

033 (5' ETS) CGCTGCTCACCAATGG

202 (U14) TCACTCAGACATCCTAGG

214 (U24) TCAGAGATCTTGGTGATAAT

403 (*PGK1*) ACCGTTTGGTCTACCCAAGTGAGAAGCCAAGACA

499 (*TSA1*) GGAGTATTCGGAGTCAGTGGAGGCGAAAAGAACT

Table S3. Other oligonucleotides used in this work.

RRP41F1 UP45: ATTTACAAAAAAACTTTAGTGCCATAACTACAGCAGGATCATCATGAATT CGAGCTCGTTTAAAC

RRP41R1 DN45: ATCGAGACGTAGCCCTTCTGGCGAGTATATTTCTAGTCTTGACATG CACTGAGCAGCGTAATCTG

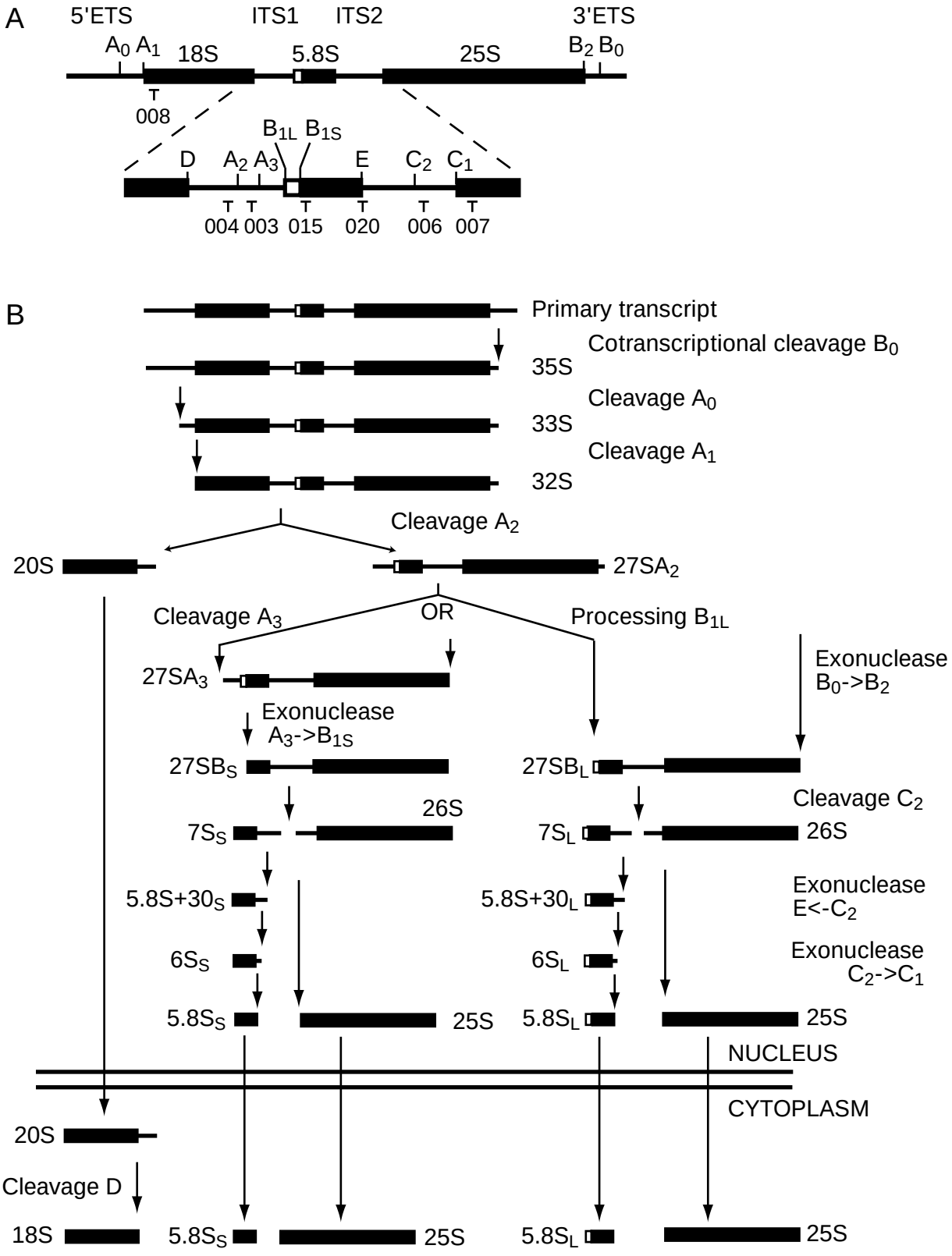
RRP44F1 UP45: AACGAGTTTTATTTATCATACTTGCATCATACAGGCCAAAACAAC
GAATTCGAGCTCGTTTAAAC
RRP44R1 DN45: ATCTGCAAGTCTCTTCCGTCTGGGGGCGATAGCGGGAACTGA
CATGCACTGAGCAGCGTAATCTG
TRF5F2 UP45: GCTCAAACGAGAAGGGACTACTGGCTCTCTAAAGGCCAGG
CTCTTTCCATGGAAAAGAGAAG
TRF5R2 DN45: TATTCTTGTATAAATAGTAAATAGTCTATAAGAGTCTATATTG
TGTACGACTCACTATAGGG
TRF5 MUT1: TTGCCGGGTTCTGCAATTGCATGTGTCGTAAAC
TRF5 MUT2: GTTTACGACACATGCAATTGCAGAACCCGGCAA

Legend to Supplementary Figure S1

A: Structure of the yeast pre-rRNA and locations of oligonucleotides used.
The 35S pre-rRNA contains the sequences of the mature 18S, 5.8S and 25S rRNAs, which are separated by internal transcribed spacers 1 and 2 (ITS1 and ITS2) and flanked by the 5 and 3' external transcribed spacers (5'ETS and 3'ETS).

B: The yeast pre-rRNA processing pathway. A complex processing pathway converts the 35S pre-rRNA primary transcript to the mature rRNAs. In wild-type cells, the 35S pre-rRNA is cleaved at site A_0 producing the 33S pre-rRNA. This molecule is rapidly cleaved at site A_1 to produce the 32S, which is cleaved at site A_2 releasing the 20S and $27SA_2$ pre-rRNAs. The 20S pre-rRNA is exported to the cytoplasm where it is cleaved at site D, by an unidentified enzyme, to generate the mature 18S rRNA. $27SA_2$ is processed via two alternative pathways. It is either cut at site A_3 to generate $27SA_3$, which is then trimmed to site B_{1S}, producing $27SB_S$. Alternatively, it can be processed to $27SB_L$ by an as yet unknown mechanism. $27SB_S$ and $27SB_L$ are matured to the 5.8S and 25S following identical pathways. Cleavage at site C_2 generates the 7S and 26S pre-rRNAs. The 7S pre-rRNA is digested 3' to 5' to 6S pre-rRNA and then to the mature 5.8S rRNA. The 26S pre-rRNA is digested 5' to 3' to the e mature 25S rRNA. During this maturation the rRNA regions will assemble with the 80 ribosomal proteins, and the pre-rRNAs will transiently associate with ~170 protein processing and assembly factors and ~70 snoRNAs.

C: Cartoons of the predicted structures of aberrant pre-rRNA species detected in *rrp6*Δ strains. The 23S and 21S RNAs are aberrant intermediates, which are generated by premature cleavage at site A_3, We have not mapped the ends of the 17S' species but analyses of other ribosome synthesis mutants identified a similar RNA that has a 3' end at site A_3 and 5' ends at heterogeneous positions within the mature 18S rRNA sequence. This may arise from inefficient 5' degradation of the 23S and 21S species.



Houseley Figure S1